inducible RYR2 expression, we found that over-expression of MG56 led to suppression of RyR2-mediated store-overload induced calcium release (SOICR) from the endoplasmic reticulum (ER). The findings suggest the possibility that MG56 functions as a stabilizer of RyR channel function. Since MBOAT family proteins contain acyltransferase activity, we hypothesize that the putative acyltransferase function for MG56 may be involved in the Ca regulatory function. In preliminary studies, we found that pretreatment of muscle fibers with 2-bromohexanoic acid, an inhibitor of palmitoyl acyltransferases, led to suppression of Ca spark activity. The effect of 2- bromohexanoic acid was also observed in HEK293 cells expressing RyR2, where SOICR activity was reduced upon treatment. Future studies are required to dissect the functional interaction between MG56 and RyR2, and to delineate the potential role for MG56-mediated lipid modification of RyR2 in the overall Ca signaling in cardiac muscle.

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Cisplatin-Induced Cachexia in rats Causes Alterations in Skeletal Muscle Calcium Homeostasis

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Cachexia is a wasting condition associated with cancer types and, at the same time, is a serious and dose-limiting effect of cancer chemotherapy. Skeletal muscle loss is the main characteristic of cachexia and the primary cause of function impairment, fatigue and respiratory complications. Because calcium homeostasis is a biomarker of muscle function, here we assessed whether it is altered in cachetic muscles by using fura-2 cytofluorimetric and electrophysiological techniques in the rat model of cisplatin-induced cachexia. We showed that besides a significant reduction of the muscle weight and fiber diameter, indexes of an overt muscle atrophy, cachetic EDL fast-twitch fibers are characterized by a significant 56% increase of resting intracellular calcium, $[Ca^{2+}]$ i, compared to control rats. Moreover the amplitude of the calcium transient induced by caffeine and depolarizing high potassium solution was significantly reduced in cisplatin-treated rats. Importantly, changes of some calcium-dependent functional outcomes, such as an increase of the latency of the action potential and a decrease of resting chloride conductance, also occurred in cachetic EDL muscles, thus indicating that the cachexia-induced alteration of calcium homeostasis influences muscle functionality. To gain insight into the molecular mechanism responsible for calcium handling alteration, we are currently investigating the expression levels of the genes related to calcium machinery in cachetic muscles. Our findings provide the first direct evidence of a calcium homeostasis dysregulation in cachexia, thus contributing to the clarification of the mechanisms underlying the muscle wasting and to the identification of druggable targets for the treatment of this invalidating syndrome.

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STIM1 Enhances SR Ca^{2+} Content through Inhibiting Phospholamban in Rat Ventricular Myocytes

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In ventricular myocytes, the physiological function of stromal interaction molecule 1 (STIM1), an ER/SR Ca^{2+} sensor, is unknown with respect to its cellular localization, its Ca^{2+}-dependent mobilization, and its action on Ca^{2+} signaling regulation. This study is seeking to determine the function of STIM1 in Ca^{2+} signaling in adult ventricular myocytes. Using freshly isolated adult rat ventricular myocytes and those in short-term primary culture and confocal microscopy, we measured real-time Ca^{2+} signaling and tracked the cellular movement of STIM1 with m-cherry and immunofluorescence. We found that endogenous STIM1 was expressed at low but measureable levels along the Z-disk, in a pattern of puncta and linear segments consistent with the STIM1 localizing to the junctional sarcoplasmic reticulum (jSR). Depleting SR Ca^{2+} using the SERCA inhibitor thapsigargin (2-10 μM) changed neither the STIM1 distribution pattern nor its mobilization rate, evaluated with fluorescence recovery after photobleaching (FRAP). Consistent with this result, native protein gel electrophoresis showed that STIM1 in heart exists mainly as a multimer, which is not altered by SR Ca^{2+} depletion. Additionally, we found no store-operated Ca^{2+} entry (SOCE) in control or STIM1 overexpressing ventricular myocytes. Nevertheless, STIM1 overexpression in these cells has a surprising phenotype with increased SR Ca^{2+} content and with increased SR Ca^{2+} leak. These changes in Ca^{2+} signaling in the SR appear to be due to STIM1-phospholamban (PLN) binding and thereby through indirect activation of SERCA2a. We therefore conclude that STIM1 contributes to the regulation of SERCA2a activity, the rate of SR Ca^{2+} leak and that these actions are independent of SOCE, a process that is absent in normal heart cells.

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Regulation of Calcineurin by Calcium-Binding Cousins

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Calcineurin (CaN), a heterodimeric, calcium-activated phosphatase (PP2B), dephosphorylates members of the NF-AT family of transcription factors. This promotes nuclear localization of NF-AT, which initiates transcription of genes controlling immune responses and regulates key steps in eukaryotic development, especially cardiac muscle. Calcium partially activates CaN by binding to CaN_B, its intrinsic calcium-binding subunit, but maximal CaN activity requires binding of calmodulin (CaM), a ubiquitous 4-EF-Hand cousin of CaN_B. The activity of CaN changes based on intermediate occupancy of the N- and C-domains of CaN_B and CaM. To probe how CaN_B controls the phosphatase activity of CaN, we compared CaN regulated by mutant forms of CaN_B in which one or more calcium-binding sites were ''knocked out''. The double knockouts in each domain reduced activity to basal level. Knockouts of individual sites within each domain were not equivalent, despite their sequence similarity and cooperativity in WT CaN. In comparison, in CaM, individual mutations of the N-domain calcium-binding sites had a larger effect on enzymatic activity of CaN than individual mutations of the C-domain sites, although the affinity of the isolated C-domain for the CaMBD is almost 10-fold higher than that of the isolated N-domain. These studies were consistent with our prior thermodynamic measurements of the affinity of the same site-knockout CaM mutants for the canonical CaMBD sequence embedded in a YFP-CFP biosensor. They support a model in which calcium binding to the N-domain of CaM is a critical trigger for the transition from medium to high activity. *Supported by AHA 12GRNT12050395 & NIH R01 GM57001.*

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Identify the Binding Interface between Calsenilin and Presenilin 1 C-Terminal Fragment

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Presenilin 1 (PS1) is the catalytic component of γ-secretase complex that cleaves β-amyloid precursor protein (APP). Mutations in PS1 alter γ-secretase activity leading to increase in production of the 42-residue amyloid-β (Aβ42) plaques found in early-onset Alzheimer's disease. Despite a few structural details known, the widely accepted topology model for PS1 N-terminal-fragment (NTF) consists of six α-helices and a transmembrane segment, whereas PS1 C-terminal-fragment (CTF) contains a membrane-spanning helix-8 (T407-F428), helix-7 (G384-A398), helix-β (E356-L369), and the flexible and solvent exposed helix-9 (F441-A461). Recent findings have suggested important functional properties of PS1-calsenilin (CSEN) complex including APP processing, Aβ42 generation, neuronal apoptosis, and calcium signaling. However, molecular details about the binding interface between PS1-CTF and CSEN as well as the impact of Ca^{2+}/Mg2+ on PS1-CSEN interactions are missing. Here we show that CSEN binds to PS1-helix-7 in a Ca^{2+} dependent manner with a dissociation constant of 9.8 μM for Ca^{2+}CSEN and 9.4 μM for Ca^{2+}/Mg2+CSEN. In contrast, very weak interaction was determined for the association of PS1-helix-7 with Mg2+CSEN (Kd = 84 μM) or ApoCSEN (Kd = 146 μM). We further found that the association between C-terminal domain of CSEN and PS1-helix-7 is Ca^{2+} dependent with a Kd of 22 μM in the Ca^{2+} bound form and no binding for the Apo-state. Interestingly, no interaction was determined for CSEN and PS1-helix-β. We have also shown that CSEN in its dimer state binds to PS1-helix-9 in a Ca^{2+} dependent manner with Kd ~ 0.8 μM for Ca^{2+} and Ca^{2+}/Mg2+ forms. Taken together, our results suggest that the major binding site between CSEN and PS1-CTF is mediated by residues on CSEN-C-terminal domain and PS1-helix-9 and a minor interaction is via hydrogen bonds between CSEN-C-terminal domain and PS1-helix-7.

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Role of Mitochondrial Ca^{2+} Uniporter in Radiation-Induced Cell Damage

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Radiotherapy for head and neck cancer induces significant acute- and long-term damage in other tissues within the treatment area, such as salivary gland.